Tutors

Dra. Núria Serrano
Departament d'Enginyeria Química i Química Analítica

Dr. Xavier Cetó
Departament d'Enginyeria Química i Química Analítica

Grau de Química

Treball Final de Grau

Dimethylglyoxime modified screen-printed electrodes for heavy metals determination

Elèctrodes serigrafiats modificats amb dimetilglioxima per a la determinació de metalls pesants

Miguel Rosal Soria

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Thanks to the Electroanalysis group of the University of Barcelona, especially to my tutors Núria Serrano and Xavier Cetó.

As well as to my family and friends for the support during all these years.

REPORT

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1. SUMMARY

Heavy metals concentration has increased due to the anthropogenic activity. As a consequence, higher concern about their potential effects on the human health and the environment has grown as they are systemic toxicants known to induce multiple organ damage, even at very low concentrations. Consequently, the contamination of natural or drinking water by heavy metals supposes a significant threat to natural ecosystems and human health, requiring methods that allow their determination and monitoring at low concentrations. Particularly, nickel is a common industrial pollutant found in wastewater, contaminated soils and in the air around industrial areas .Nickel toxicity in humans has received important attention due to the carcinogenic character of this element.

The conventional methodology for the determination of heavy metals is based on the use of atomic spectroscopic techniques. However, these systems are expensive, bulky and require qualified personnel capable of carrying out the analyses. In addition, they do not usually allow the analysis of the samples on-site. For the on-site detection of heavy metals, electrochemical devices offer attractive possibilities to meet these needs. The advantages of electrochemical systems include high sensitivity and selectivity, high speed, a broad linear range, compatibility with modern micro fabrication techniques, low energy and space requirements and low cost instrumentation. In addition, both the sensor and the instrumental part can be easily miniaturized to obtain compact portable devices that are easy to use on-site.

In this regards, this work presents the development of a voltammetric sensor based on carbon screen-printed electrodes (SPCEs) chemically modified with dimethylglyoxime (DMG) for the determination of Ni(II) by adsorptive stripping voltammetry (AdSV). Developed sensors were analytically characterized in terms of sensitivity, selectivity, limits of detection and quantification, linear range, repeatability, reproducibility and durability. Lastly, the developed sensor was applied to the determination of Ni(II) in a certified wastewater sample.

Keywords: Dimethylglyoxime, screen-printed electrode, adsorptive stripping voltammetry, nickel.

2. RESUM

La concentració de metalls pesants ha augmentat a causa de l'activitat antropogènica. Com a conseqüència, s'ha intensificat la preocupació pels seus efectes nocius sobre la salut humana i al medi ambient. La toxicitat dels metalls pesants és capaç de produir danys als òrgans humans, fins i tot a concentracions molt baixes. Com a conseqüència, la contaminació de l'aigua natural o potable amb metalls pesants suposa una amenaça important per als ecosistemes naturals i la salut humana, que requereixen mètodes que permetin la seva determinació a baixes concentracions. Particularment, el níquel és un contaminant industrial comú que es troba en aigües residuals, als sòls i a l'aire al voltant d'àrees industrials. La toxicitat del níquel en humans ha rebut especial atenció a causa del seu caràcter cancerigen.

La metodologia convencional per a la determinació de metalls pesants es basa en l'ús de l'espectroscòpia atòmica. No obstant, aquests sistemes són cars, voluminosos i requereixen personal qualificat capaç de dur a terme els anàlisis. A més, no solen permetre l'anàlisi de mostres *in situ*. Per a la detecció *in situ* de metalls pesants, els dispositius electroquímics ofereixen atractives possibilitats per satisfer aquestes necessitats. Els avantatges dels sistemes electroquímics inclouen alta sensibilitat i selectivitat, alta velocitat, un ampli rang lineal, compatibilitat amb les tècniques modernes de microfabricació, baixos costos energètics i instrumentació de baix cost. A més, tant el sensor com la part instrumental es poden miniaturitzar fàcilment per obtenir dispositius portàtils compactes fàcils d'usar *in situ*.

Aquest treball presenta el desenvolupament d'un sensor voltamperomètric basat en elèctrodes serigrafiats de carboni (SPCEs) modificats químicament amb dimetilglioxima (DMG) per a la determinació de Ni(II) per voltamperometria d'adsorció per redissolució (AdSV). El sensor es va caracteritzar analíticament en termes de sensibilitat, selectivitat, límits de detecció i quantificació, interval de linealitat, repetibilitat, reproductibilitat i durabilitat. Finalment, el sensor es va aplicar a la determinació de Ni(II) en una mostra certificada d'aigües residuals.

Paraules clau: Dimetilglioxima, elèctrodes serigrafiats, voltamperometria d'absorció per redissolució, níquel.

3. INTRODUCTION

Heavy metals such as cadmium, lead, mercury, arsenic, chromium, copper, manganese and nickel have been associated with contamination and potential toxicity or ecotoxicity. Heavy metals may enter the body in food, water, air, or by absorption through the skin, and they are especially dangerous because they tend to bioaccumulate. Particularly, nickel is a common industrial pollutant with concentrations in wastewater around industrial areas, in contaminates soils and in the air of industry areas. Nickel toxicity in humans has received important attention due to the carcinogenic character of this element.[1]

Stripping techniques are especially suitable for the determination of metal ions traces in samples of environmental origin due to their low detection limits and sensitivity to different metallic species. Historically electrodes used were based on mercury, gold, platinum and carbon, being the mercury based-electrodes the most common ones. Currently, although European regulations don't prohibit the use of mercury electrodes, there are pressures to replace them.[1]

The complexation of Ni(II) by dimethylglyoxime (DMG) has long been used as the basis of one of the most selective gravimetric determinations available with only a few metallic species showing any competing tendency to form a DMG complex. Therefore, it was expected that a highly selective chemical pre-concentration of Ni(II) could be achieved with a chemical modified electrode (CME) based on DMG-containing carbon paste and produce a simple mercury free electrode with properties useful for practical Ni(II) determination.

It is possible to take advantage of the well-known use of DMG as complexing agent for metal ions such as nickel, cobalt, palladium, etc. To develop screen-printed electrodes modified with DMG for the determination of heavy metals, upon optimization of the experimental conditions.

3.1. HEAVY METALS

The term heavy metal is commonly used to refer to a group that mainly includes transition metals, some semimetals, lanthanides and actinides that have been associated with pollution and potential toxicity or ecotoxicity, although has never been defined by any authoritative body such as IUPAC.[2]

A more standardized definition is that heavy metals are those whose density is at least five times greater than that of water. The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. The terms heavy metals and toxic metals can be used as synonyms although only some of them belong to both groups.[3]

The most important heavy metals are: Arsenic (As), Cadmium (Cd), Cobalt (Co), Iron (Fe), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), Tin (Sn) and Zinc (Zn).

Although some of them are necessary for humans, biological processes or industry, in high concentrations they can be dangerous. One of the biggest problems associated with the appearance of heavy metals is related to their bioaccumulation and/or biomagnification causing a greater exposure of these metals to an organism compared to the levels found in the environment.[4]

The continuous human exposure to heavy metals constitutes a risk for the public health, mainly in developing countries. The heavy metals are ubiquitous air and water pollutants and are associated with multiple adverse health effects. The main organs affected by metals are kidneys, lungs, liver, bowels and haematological system, but mainly the peripheral and central nervous systems. The damage severity depends on the time and levels of exposure, the rate of absorption, the individual susceptibility and the route through which the metal is absorbed. A variety of mechanisms have been attributed to heavy metal-induced toxicity and they have often been related with the generation of reactive oxygen species and depletion of antioxidant defence enzymes, producing an increase of cellular oxidative stress. [5]

Table 1 shows the effects of the toxicity of heavy metals in humans and the concentration at which the symptoms begin to appear.

Metal	Concentration (mg/L)	Effects
Arsenic	0.5	Lung, liver and skin cancer; teratogenic; poison at high doses.
Cadmium	0.2	Accumulation in kidneys, lungs and heart; carcinogenic; poison at high doses.
Chromium	1.0	Lung cancer.
Iron	10.0	Siderosis (lung disease).
Lead	0.15	Brain damage (especially in children); anaemia; paralysis.
Mercury	0.05	Attacks the central nervous system; neurological effects; effects on the immune system.
Nickel	1.0	Lung cancer; allergic reactions on the skin.
Vanadium	0.5	Bronchial spasms; emphysema.
Zinc	5.0	Fever; nausea; muscle aches.

Table 1. Toxicity effects of heavy metals according to concentration.[6]

3.1.1. Nickel

In particular, this research will tackle the determination of nickel, so from now on we will focus on this metal. The most important characteristics are those related to its toxicity and its impact on the environment.

According to Environmental Protection Agency (EPA) and Agency for Toxic Substances and Disease Registry (ATSDR), the health effects due to the exposure to any hazardous substance will depend on the dose, duration and manner of exposure, characteristics and personal habits, and whether other chemicals are present. Particularly nickel can be released into the environment through the chimneys of large furnaces used to make alloys or by power plants and garbage incinerators, as well as into industrial wastewater.[7]

The nickel produced by the chimneys of power plants sticks to small dust particles that are deposited on the ground or removed from the air on rain or snow. A large amount of nickel released into the environment ends up in the soil or in sediment where it adheres strongly to iron or manganese-containing particles. Acidic conditions help the mobilization of nickel in the soil and facilitate its filtration into groundwater. Therefore, possible exposure pathways are: direct inhalation through the mouth and nose, dermal absorption, and indirect ingestion via contaminated food and drinks.[7]

Nickel is often considered because of its poisoning potential. However, nickel contact has also unfavourable consequences to humans, such as dermatitis and carcinogenesis. Those rely on active changes in metabolism, inflammation, oxidative stress, cell proliferation and cell death. Despite its poisoning potential, nickel plays fundamental biological roles in plants, bacteria, archaea, and unicellular eukaryotes, making it a double-faced element for life. Although nickel-containing enzymes were not found in higher animals, this metal ion was classified as a "possibly essential element" for animals and humans.[8]

Various studies carried out to investigate the exposure to incinerator emissions showed the emission of heavy metals, including nickel, to the environment. Furthermore, observing a relationship between the distance from emissions and the concentration of heavy metals in the environment, soil and living beings. Notwithstanding the significant emission reduction of modern plants due to the implementation of technologies that fulfil the emission standards, there is still concern about the impact on human health.[9]

In other studies, areas influenced by traffic and nearby industrial activities were assessed the human health hazards associated with heavy metals, including nickel.[10] Air pollution may be the cause of a variety of adverse effects on human health, such as heart disease, lung cancer, acute respiratory infections in children and chronic bronchitis in adults. Air pollution can also aggravate pre-existing heart and lung disease and trigger asthmatic attacks. Furthermore, the International Agency for Research on Cancer (IARC), which is the specialized cancer agency of the World Health Organization (WHO), classified outdoor air pollution as carcinogenic to humans.[10]

Although lung cancer cases are attributable in part to smoking tobacco, environmental pollution is the most common cause for non smoking-related lung cancers. Nickel is one of the most common elements that can be found on the environment, and it is considered as a carcinogen and co-carcinogen by the IARC.[11] Carcinogens are defined as a pollutant that can

increase the risk of cancer, whereas co-carcinogens are chemical substances that cannot induce cancer when they are administered alone, but can enhance the carcinogenic effect of other substances..[12] It has been discovered that exposure to nickel promotes the malignant transformation of human bronchial epithelial cells.[11] Moreover nickel has an important role in carcinogenesis of colonic cancer.[13] For humans, the daily uptake of nickel via diet is more than 3 times the recommended daily supply and the 80–90% is excreted.[13] The quantity of Ni absorbed by the gastrointestinal tract depends on the Ni species and the total Ni content in the food, and also on the individual absorption capacity.[13]

Urbanization and industrialization affect urban environments in a unique way. Of particular concern is the health risk associated with heavy metals, such as nickel content of the soils. The plants take their nutrients from those soils, and the toxic compounds present in the soil can be incorporated to the plants, and later on to humans upon their ingestion. Although nickel acts as a micronutrient, necessary for plant survival and its growth, it is toxic in excess.[14] The urban soil contamination by heavy metals remains as a topical issue because of the risks that represent to human health. The upper layers of soil near the smelting plants have high levels of heavy metals contamination.[15]

Lastly, another form of exposure to nickel would be water consumption. According to the directive of the European Community on the quality of water for human consumption, the concentration of nickel cannot exceed 20 µg/L, although this value tends to increase in rivers near industrial zones[16]

3.2. ELECTROANALYTICAL METHODS

Nickel is generally found at very low concentration levels on the environment; therefore, very sensitive methods are required to detect nickel in environmental samples. Although there are methods with adequate sensitivity for the determination of heavy metals such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) and inductively coupled plasma mass spectrometry, electrochemical methods are one of the most favourable techniques for determination of heavy metal ions and represent an attractive alternative to those given their high sensitivity, low cost and portability. Electroanalytical methods, in particular voltammetry, are very suitable for the determination of metal ions at such low concentrations, since unlike

other techniques with also very low detection limits, they are also sensitive to the presence of different metallic species. Figure 1 compares the detection limits of electroanalytical methods with those of other analytical techniques.[17]

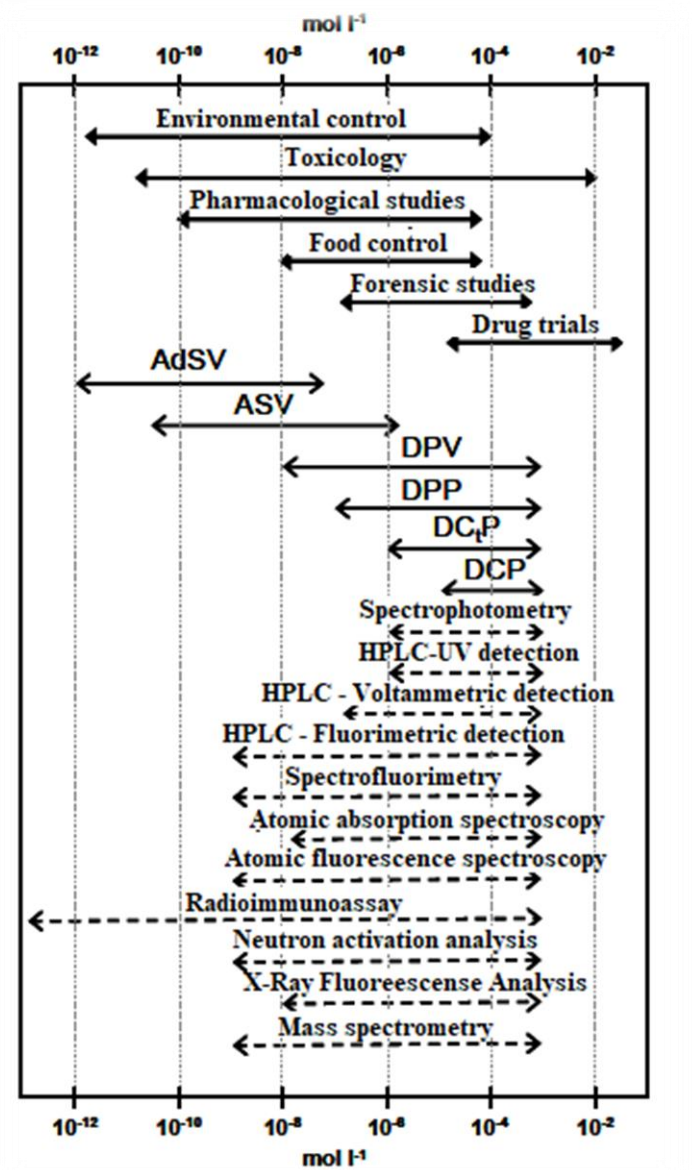


Figure 1. Limit of detection of different analytical techniques.[16]

Among all the electroanalytical methods, voltammetries are a group of techniques that have been used for the determination of metal ions at low concentration levels. These techniques have a high sensitivity and selectivity, a great capacity to differentiate between different chemical species, an instrumental simplicity and a low cost. These advantages make them especially interesting for the determination of heavy metals.[18]

3.2.1. Stripping techniques

Stripping techniques have a first pre-concentration step, where the metal is accumulated on the electrode by the application of a constant potential under stirring. To achieve good and reproducible results it is necessary to optimize and control the hydrodynamic parameters (pre-concentration time and potential, stirring, temperature, electrode area and the applied initial potential). The application of this pre-concentration step provides an increase of sensitivity of about 2 or 3 orders of magnitude, making it feasible to operate with analyte concentrations down to 10^{-10} mol/L or even lower. The second step consists of a rest period, which is applied after the pre-concentration step keeping the potential constant but without stirring. During this period is achieved the homogenizing of analytes on the electrode surface. Finally, the stripping step in which the deposited analyte is determined by the application of a potential sweep. A number of different waveforms have been used for the stripping step, including linear sweep voltammetry (LSSV), differential pulse voltammetry (DPSV), and square wave voltammetry (SWSV). SWSV and DPSV are more commonly used, due to their lower detection limits.[19]

The stripping techniques have lower detection limit than any of the commonly used electrochemical techniques, and the preparation of the sample is easy, in addition the sensitivity and the selectivity are excellent. Also, they allow detecting concentrations of heavy metals in the mg/L range (single digits), this levels range allows to be within the maximum limits for human consumption according with the World Health organization (WHO), which leads to the conclusion that it is a promise by its low cost, high sensitivity and diversity.

Stripping voltammetric techniques include, among others, the anodic stripping voltammetry (ASV) and the adsorptive stripping voltammetry (AdSV).

3.2.2. Anodic stripping voltammetry (ASV)

For heavy metal ions determination, anodic stripping voltammetry (ASV) is the most used stripping technique. In the first pre-concentration step, a cathodic electrodeposition of the analyte or analytes from the solution is carried out on the surface of the working electrode, applying a controlled potential for a determined time. The deposition potential is normally 0.5 V more negative than the metal reduction potential (or of the more easily oxidizable metal if various are present). This pre-concentration step is carried out with stirring and the mass transport is produced by diffusion and convection. The second step is the rest, where the same deposition potential is applied but without stirring. Finally, the last step is the stripping, in which the accumulated analytes on the electrode is totally or partially redissolved, applying an anodic potential sweep and producing a signal in peak form. The voltammetric signal depends on several parameters related to pre-concentration and redissolution steps, the characteristics of the metal ion and the geometry of the used electrode.[18]

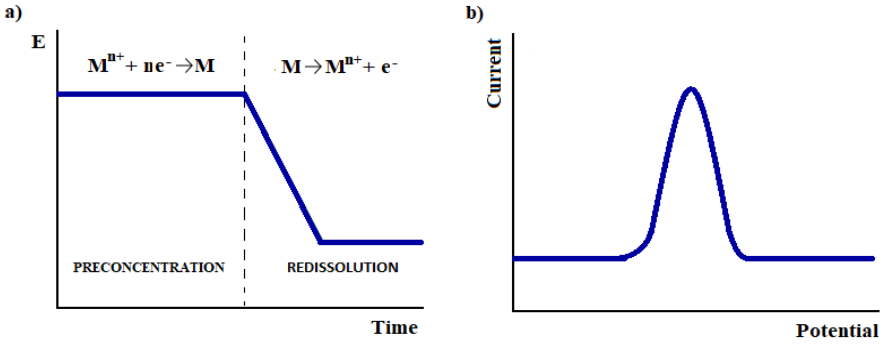


Figure 2. Anodic stripping voltammetry: **a)** potential-time sequence, **b)** typical voltammogram

The instrumental parameters that must be controlled during the pre-concentration step are the deposition potential, the deposition time, the stirring speed and the size of the drop if a hanging mercury drop electrode (HMDE) is used. The deposition potential must be more negative as the redox potential of the metal deposited decreases. Regarding the deposition time, the longer the deposition time applied, the greater the amount of analyte deposited on the surface of the electrode. However, too long deposition times can lead to the formation of intermetallic compounds or the saturation of the electrode. The agitation favours the pre-

concentration step because it facilitates the convective transport of the ions towards the electrode.[18]

3.2.3. Adsorptive stripping voltammetry (AdSV)

Adsorptive stripping voltammetry (AdSV) greatly improves the number of analytes than can be measured by voltammetry at trace levels. This is achieved due to the adsorption time, since the amount of analyte deposited in the working electrode is proportional to the adsorption time. In the case of metal ions, this strategy involves the formation, adsorptive accumulation, and reduction of an electroactive complex of the metal on the surface of the electrode. In this type of stripping voltammetry, the reduction of the metal of the adsorbed complex are produced (although it is also possible to analyze the reduction of the ligand) by means of the application of a cathodic potential sweep. The response of the adsorbed species on the electrode surface is directly related to its surface concentration and with the adsorption isotherm. The maximum adsorption density is related to the size and surface concentration of the adsorbed complex.[20]

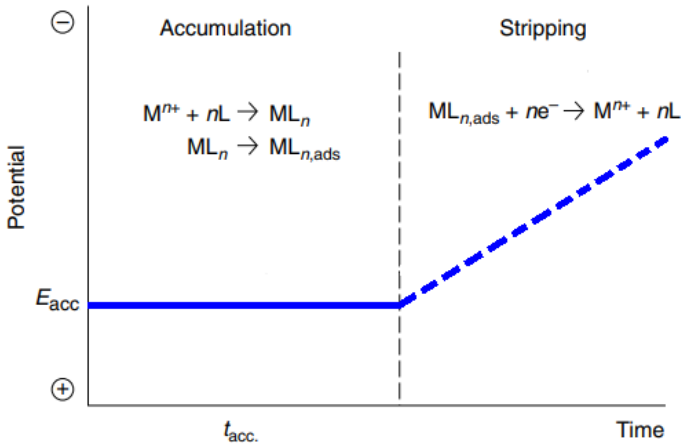


Figure 3. Accumulation and stripping steps in adsorptive stripping measurements of a metal ion (M^{n+}) in the presence of an appropriate chelate agent (L).

Short adsorption times (1–5 min) result in a very effective interfacial accumulation. The reduction step is also very efficient as the entire accumulated complex is reduced. Such a combination results in extremely low detection limits (10^{-10} – 10^{-11} mol/L) for the determination

of metals, including uranium, vanadium, aluminium, or molybdenum; or even lower detection limits (10^{-12} mol/L) for platinum, titanium, cobalt, chromium, or iron, which can be measured by coupling the adsorption accumulation with catalytic reactions. In this case, the response of the accumulated complex is greatly amplified through a catalytic cycle, for example, in the presence of an oxidant. The adsorptive approach may also offer improvements in selectivity and/or sensitivity for metals (for example tin or nickel) that are measurable also by conventional stripping analysis. Examples of AdSV schemes for measuring trace metals are listed in Table 2. All procedures rely on an accurate choice of the chelating agent. The resulting complex should be surface active and electroactive. In addition, selective complexation can be used to enhance the overall selectivity.[20]

Metal	Complexing Agent	Supporting Electrolyte	Detection Limit (mol/L)
Al	Di(hydroxyanthraquinone sulfonic acid)	BES buffer	1×10^{-9}
Be	Thorin	Ammonia buffer	3×10^{-9}
Co	Nioxime	HEPES buffer	6×10^{-12}
Cr	Diethylenetriamine-pentaacetic acid	Acetate buffer	4×10^{-10}
Fe	Solochrome violet RS	Acetate buffer	7×10^{-10}
Mn	Eriochrome Black T	PIPES buffer	6×10^{-10}
Mo	Oxine	Hydrochloric acid	1×10^{-10}
Ni	Dimethylglyoxime	Ammonia buffer	1×10^{-10}
Pt	Formazone	Sulfuric acid	1×10^{-12}
Sn	Tropolone	Acetate buffer	2×10^{-10}
Ti	Mandelic acid	Potassium chlorate	7×10^{-12}
U	Oxine	PIPES buffer	2×10^{-10}
V	Catechol	PIPES buffer	1×10^{-10}

Table 2. Common AdSV schemes for measurements of trace metals[20]

3.3. ELECTROCHEMICAL CELL

The cell is usually a covered beaker of 5–50mL volume, and contains three electrodes (working, reference, and auxiliary), which are immersed in the sample solution. While the working electrode is the electrode at which the reaction of interest occurs, the reference electrode provides a stable and reproducible potential (independent of the sample composition), against which the potential of the working electrode is compared. Such "buffering" against potential changes is achieved by a constant composition of both forms of its redox couple, such as Ag/AgCl or Hg/Hg_2Cl_2, as common with the silver–silver chloride and the saturated calomel reference electrodes, respectively. To minimize contamination of the sample solution, the reference electrode may be insulated from the sample through an intermediate bridge. An inert conducting material, such as platinum wire or graphite rod, is usually used as the current-carrying auxiliary electrode. The relative position of these electrodes and their proper connection to the electrochemical analyzer should be noted.[20]

Glass is commonly used as the cell material, due to its low cost, transparency, chemical inertness and impermeability. Teflon and quartz represent other possible cell materials. The cell cover can be constructed of any suitable material that is inert to the sample. An accurate temperature control is readily achieved by immersing or jacketing the cell in a constant-temperature bath.[20] Figure 4 shows a schematic diagram of an electrochemical cell.

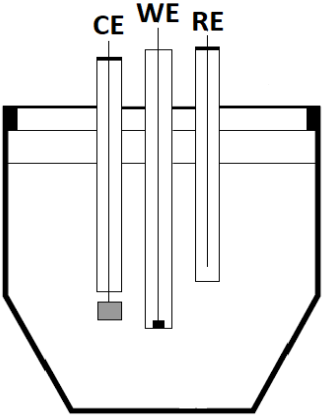


Figure 4. Schematic diagram of a cell for voltammetric measurements: working electrode (WE); reference electrode (RE); counter electrode (CE)

3.4. WORKING ELECTRODES

The performance of the voltammetric procedure is strongly influenced by the working-electrode material. The working electrode should provide high signal-to-noise characteristics, as well as a reproducible response. Thus, its selection depends primarily on two factors: the redox behaviour of the target analyte and the background current over the potential region required for the measurement. Other considerations include the potential window, electrical conductivity, surface reproducibility, mechanical properties, cost, availability and toxicity. A range of materials have been used as working electrodes for electroanalysis.[20] Hanging mercury drop electrode (HMDE) and the mercury film electrode (MFE) were typically used for the determination of metal ions by stripping techniques, particularly in the cathodic side; while solid electrodes (Au, Pt, vitrified carbon or carbon paste) were indicated for the study of anodic processes. Nowadays, studies are directed more towards these last ones.[17]

3.4.1. Mercury electrodes

Mercury based electrodes have been particularly of interest for the analysis of metal ions by voltammetry and particularly it should be pointed out their excellent performance with stripping voltammetry. These mercury based electrodes are the HMDE, the dropping mercury electrode (DME/SMDE) and the MFE. Main advantages of these classical mercury devices are its high sensitivity and reproducibility, high hydrogen over potential, wide cathodic potential range (from 0.4 V to -2.5 V depending on the supporting electrolyte), the possibility to form amalgams and facility to obtain pure surfaces. However, because of mercury toxicity, new European regulations devoted to the banning of exports and safe storage of metallic mercury have appeared in the last decades, which have caused a policy against this material that has led to its almost total disappearance in most products or processes. Although in the case of voltammetry, the use of mercury is not forbidden, this limitation, instead of reducing the use of voltammetry as an analytical technique, has encouraged researches to explore alternative electrode materials with features similar to those of mercury, but with lower toxicity.[21,22]

3.4.2. Screen-printed electrodes (SPEs)

Nowadays screen-printing technology is a recognized method for the fabrication of voltammetric sensors and biosensors for several applications, including the determination of metal ions. Screen-printed electrodes (SPEs) usually include a three electrode configuration (working, counter and reference electrodes) printed on the same strip.[1]

Their popularity has arisen due to their distinct advantages when compared to traditional noble metal based electrodes, such as being comparatively cheap and easily obtainable, whilst out-performing traditional metals with their structural polymorphism, chemical stability, wide operable potential windows and relatively inert electrochemistry.[23]

The advent of screen-printing technology has opened new opportunities for the application of electrochemical techniques for environmental analyses outside a laboratory. SPEs may be the most appropriate electrochemical sensors for *in situ* analysis because of their linear output, the integration of the three electrodes in a single sensor, the small volume of sample needed, low power requirement, quick response, high sensitivity and ability to operate at room temperature. In addition, the surface of SPEs can be easily modified to fit the detection of different analytes and/or to achieve an improvement of its analytical performance. Many kinds of modifiers for SPEs have been reported, including noble metals, alternative carbon sources such as graphene and graphite[23], inorganic nanomaterials, biorecognition elements such as enzymes and DNA sequences, etc.. The printing process of SPEs is carried out on a ceramic or polymer plate. The ink most commonly used is the carbon ink, as it is a widespread material, relatively inexpensive, easy to modify and chemically inert. Gold and platinum inks are also quite employed in SPEs, but not as much as carbon because of its higher cost.[24]

Figure 5. Screen-printed electrode DRP-C110 manufactured by DropSens

3.5. VOLTAMMETRIC DETERMINATION OF NICKEL

Although electroanalytical stripping techniques allow the quantitation of many trace metals, in the case of the determination of Ni(II) by ASV there are some difficulties involved. Numerous early attempts have shown that the Ni(II) reduction is irreversible, requiring a deposition potential of at least -1200 mV. Furthermore, the reduced nickel shows a strong tendency to form intermetallic compounds with other metallic species co-deposited at the very negative potentials required.[25] Since this reaction yields complex, matrix-dependent stripping patterns, the stripping procedure is not well suited for the quantitation of nickel (II).

Furthermore, the electrode processes for Ni(II) at mercury electrodes are far from ideal, in particular because nickel is only sparingly soluble in mercury. Thus, stripping methods for Ni at such electrodes are not sufficiently sensitive for environmental investigations. A number of studies have therefore been focused on the development of alternative electrochemical methods for Ni(II) determination.

As explained in previous sections, AdSV was used in the stripping determination of those metal ions that cannot form intermetallic compounds or amalgams with the working electrode. In this project an *ex-situ* antimony electrode obtained from the modification of a commercial screen-printed carbon electrode (Ex-situ SbSPCE) is applied to the determination of Ni(II) by AdSV and using DMG as complexing agent for Ni(II) in a solution prepared with Nickel standardized pattern.[1] In addition, nickel will be determined with the preparation of DMG modified SPEs.

These methods rely on its stripping at chemically modified electrodes (CME's) based on accumulation. CME's have chemically selective groups (modifiers) attached to their surfaces, that are specifically designed for the ability to react with and bind the target solute, and many are based on carbon or graphite such that the use of mercury is avoided. With CME's the pre-concentration is achieved by a purely non-electrochemical deposition step, following which quantitation of the analyte accumulated at the surface can be carried out by usual voltammetric measurement.

By doing so, two analytical advantages in addition to the sensitivity enhancement inherent in the pre-concentration are achieved. First, the electroanalytical quantitation might be extended to analytes that cannot be reductively deposited or which require excessively negative deposition potentials. Second, the selectivity of the chemical step used for the pre-concentration might improve the overall selectivity of the entire analysis.

Particularly in the analysis of Ni, some CMEs have already been reported consisting of carbon paste mixtures containing appropriate amounts of DMG coated onto the graphite particles by a simple dissolution/evaporation procedure. The reason for choosing this approach was the likelihood of producing a simple mercury free electrode with properties useful for practical Ni(II) determination, taking advantage of complexation of Ni(II) by DMG, since nickel is one of the few metallic species showing a competing tendency to form a DMG complex. Therefore, it was expected that a highly selective chemical pre-concentration of Ni(II) could be achieved with a CME based on DMG-containing carbon paste. Furthermore, the solubility of both DMG and its Ni(II) complex is sufficiently low to render the incorporation of the modifier onto the electrode surface and retention of the complexed analyte straightforward. Compared to other types of CMEs, the carbon paste electrodes are extremely simple to prepare, and the surface coverage of modifier is easily adjusted. In addition, a fresh CME surface is easily generated in a reproducible manner. Consequently, the carbon paste CMEs are particularly attractive for analytically oriented applications.[25–30]

In this study, the determination of Ni(II) will be carried out using the new developed DMG modified SPE. The obtained results will be compared with those achieved using an Ex-situ antimony screen-printed carbon electrode (Ex-situ SbSPCE) by AdSV using DMG as complexing agent[1].

4. OBJECTIVES

The main objective of this work is to achieve the determination of nickel in environmental samples using a dimethylglyoxime (DMG) modified screen-printed electrodes.

To achieve this, it will be necessary to:

- The development of a voltammetric sensor based on carbon screen-printed electrodes (SPCEs) chemically modified with dimethylglyoxime (DMG) for the determination of Ni(II) by adsorptive stripping voltammetry (AdSV).
- The analytical characterization of the developed sensors in terms of sensitivity, selectivity, limits of detection and quantification, linear range, repeatability, reproducibility and durability.
- The application of the developed sensor to the determination of Ni(II) in a certified wastewater sample.

5. EXPERIMENTAL SECTION

5.1. REAGENTS AND SOLUTIONS

The solutions used during this study were prepared from the following analytical quality reagents (Table 3).

Name	Formula	Commercial Distributor
Antimony pattern solution	Sb (III) 1000 mg/L	Merck
Nickel pattern solution	Ni(II) 1000 mg/L	Alfa Aesar
Hydrochloric acid	HCl 30%	Merck
Ammonia solution	NH_3 25%	Merck
Ammonium chloride	NH_4Cl	Probus S.A.
Dimethylglyoxime	$CH_3C(=NOH)C(=NOH)CH_3$	Merk
Acetone	CH_3COCH_3 99+%	Acros Organics
Ethanol absolute	CH_3CH_2OH	Panreac

Table 3. Reagents used in the experimental part

All solutions have been prepared with ultra pure water obtained with Mili-Q plus 185 water purification equipment manufactured by Millipore.

Solution of dimethylglyoxime in acetone with concentrations 0.005, 0.01 and 0.02 mol/L

Solution of dimethylglyoxime in ethanol with concentrations 0.01 mol/L

Solution of hydrochloric acid 0.1 and 1 mol/L

Buffer solution of ammonia and ammonium chloride at pH = 9.2

5.2. INSTRUMENTATION

Voltammetric measurements have been carried out with a potentiostat Autolab PGSTAT12 (EcoChemie, The Netherlands), which is connected to a stand 663 VA Stand (Metrohm, Swiss) and to a computer that has the data acquisition program GPES version 4.9 (EcoChemie, The Netherlands). The support team 663 VA Stand contains an electrochemical cell of work with the different electrodes. As electrodes, a SPEs DRP-C110 (DropSens, Spain) were used as working electrode and a combined electrode Pt 52 61 (Crison, Spain) formed by a platinum electrode and a reference electrode Ag / AgCl.

The pH measurements were made with a combined electrode Orion8103SC (Crison, Spain) connected to a pH meter Crison micro pH 2000 (Crison, Spain). The additions of the different solutions have been made with micropipettes (Nichiryo Nichipet, Japan). The sensors have been prepared using an oven 2000200 (J.P. Selecta, Spain). The instrumentation used is shown in figure 6.

Figure 6. a) Autolab PGSTAT12; **b)** 663 VA Stand; **c)** combined electrode Pt 52 61; **d)** SPE DRP-C110; **e)** pH meter Crison micro pH 2000; **f)** oven 2000200.

5.3. EXPERIMENTAL METHODOLOGY

The methodology used in the determination of nickel carried out during this study is based on the findings from previous works [27-31] although the optimal conditions for the determination have been obtained experimentally and will be explained below.

DMG-coated SPEs were prepared by drop-casting of a DMG solution in acetone on to the carbon working electrode surface. The DMG is added dropwise to a volume of 225 μL distributed in nine additions of 25 μL of a solution of DMG in acetone of 0.02 mol/L

concentration. Electrodes were allowed to dry between every deposition in an oven at a temperature of 25ºC, and rinsed with water at the end. Prior to its usage, electrodes were activated by dipping them in a Ni(II) solution with a concentration of 1 μmol/L for 5 seconds, then dipped in a 1 mol/L HCl solution for five seconds to remove the bound Ni and rinsed with water. This cycle of activation is repeated a total of three times. Finally, electrodes were rinsed with water again and ready to use.

All the above conditions were experimentally optimized through a D-optimal design, in which the amount and concentration of DMG, the temperature, the activation cycles and conditions and the rest time were the factors included in the design of experiments, whereas the voltammetric response as well as its RSD were the responses considered for the response surface modelling (RSM). The choice of a D-optimal design was preferred as it is especially suitable for RSM and it will provide a more affordable number of runs in comparison to any classical design. For the optimization process MODDE® software from Umetrics (Umeå, Sweden) was used. Finally, optimum theoretical conditions were assessed experimentally to confirm those matched with the expected performance.

Developed sensors were analytically characterized in terms of sensitivity, selectivity, limits of detection and quantification, linear range, repeatability, reproducibility and durability. Lastly, the developed sensor was applied to the determination of Ni(II) in a certified wastewater sample.

To carry out Ni(II) determination, adsorptive stripping voltammetry (AdSV) was the chosen voltammetric technique. AdSV measurements were carried out at ammonia buffer at pH 9.2, and comprised three steps: accumulation, measurement and cleaning. Firstly, electrode was immersed in a stirred cell containing 25 mL of sample for 60 s at 0 V to allow the Ni(II)-DMG interaction. Afterwards, reduction of Ni(II) adsorbed onto electrode surface took place by differential pulse voltammetry (DPV) scanning the potential from -0.7 to -1.25 V. Finally, electrode was regenerated by immersing it in a stirred cell containing 0.1 mol/L HCl for 30 s.

6. RESULTS AND DISCUSSIONS

As previously mentioned, the objective of this study is the optimization of the experimental parameters of the sensor preparation, such as the volume and the concentration of DMG added, the conditions of drying, activation or measurement, as well as its posteriori characterization. Characterization of the developed sensors will be carried out in terms of sensitivity, selectivity, limits of detection and quantification, linear range, repeatability, reproducibility and durability. Lastly, the developed sensor will be applied to the determination of Ni(II) in a certified wastewater sample.

6.1. OPTIMIZATION OF SENSOR'S PREPARATION AND MEASURING CONDITIONS

All sensor conditions were experimentally optimized through a D-optimal design, in which the amount and concentration of DMG, the temperature, the activation cycles and conditions and the rest time were the factors included in the design of experiments, whereas the voltammetric response as well as its RSD were the responses considered for the response surface modelling (RSM).

Final design comprised a total of 54 runs, which required the fabrication of different sensors under each of the conditions plus its analytical characterization. Furthermore, all experiments were done in duplicate. Afterwards, response towards a 50 μg/L Ni(II) solution was evaluated for each of the modified SPEs as described in section 5.3.With this data, the RSM was built and the optimal conditions calculated. Finally, the study of voltammetric measurements corroborated the optimal conditions obtained by the D-optimal design.

The conditions used as factors included in the D-optimal design are shown in table 4.

V DMG (μL)	C_{DMG} (mol/L)	T^a drying (ºC)	Activation Cycles	C_{Ni} (μmol/L)	Activation Time (s)	Rest Time (h)
25 - 225	0.005 - 0.02	25 - 160	3 - 12	1 - 10	5 - 60	0 - 24

Table 4. Conditions used by the D-optimal

Finally, the optimized conditions obtained were: use a volume of 225 μL of DMG with a concentration of 0.02 mol/L, a drying temperature of 25 ºC, use 3 cycles of activation, a nickel concentration of 1 μmol/L, an activation time of 5 seconds and use them the same day of preparation.

During the optimization of the preparation of the sensors, absolute ethanol was also used as solvent for DMG solution instead of using acetone. The result was that the sensors prepared with absolute ethanol as solvent did not give a replacement signal, however, those prepared with acetone did give a signal. Thus, the usage of ethanol was discarded.

Another aspect to consider in the preparation of the sensors is the time elapsed between additions and the volume of every addition of DMG solution.

The preparation of the sensor can be done in two ways:

Method 1: 9 additions of 25 μL (DMG solution) every 15 minutes between additions.

Method 2: 3 additions of 75μ L (DMG solution) every 30 minutes between additions.

During the characterization of the developed sensor will be studied which preparation method gives a better response.

6.2. CHARACTERIZATION OF SENSOR'S RESPONSE/PERFORMANCE

6.2.1. Repeatability and reproducibility

The first aspect with which the sensors have been characterized is repeatability and reproducibility. To study the repeatability, five measurements were made for each sensor and to study the reproducibility the measurements were made for three sensors prepared in the same way. The study was carried out according to the preparation of method 1 and method 2. Once the different voltammograms were obtained, the area of the peaks corresponding to Ni(II) was measured and the average, the standard deviation and the RSD (%) were calculated. The sample solution consists of 25 ml of ammonia buffer and 50 μg/L of Ni(II). Table 5 shows the results obtained for the different sensors.

	Area average	Standard deviation	RSD (%)
Method 1	1.6	0.2	10.2
Method 2	0.85	0.09	10.3

Table 5. Data from the repeatability and reproducibility experiments obtained using a DMG modified screen-printed electrode at pH = 9.2 and 50 μg/L Ni(II).

The results show that the sensors prepared by method 1 give a greater signal than the sensors prepared by method 2. Regarding terms of standard deviation and RSD, the two methods provide similar results.

An important fact regarding the repeatability of the measurements of the sensors is that during the experiments carried out, up to 20 optimal measurements of a single sensor have been obtained.

6.2.2. Sensitivity and limits of detection (LOD) and quantification (LOQ)

The sensitivity of the method determines the sensor capacity to discriminate between small variations of the analyte concentration, and corresponds to the slope of the calibration line. The LOD is defined as the minimum concentration or quantity of analyte that can be detected and can be calculated as three times the standard deviation of the ordinate divided by the slope. The LOQ is defined as the minimum concentration or quantity of analyte that can be quantified and can be calculated as ten times the standard deviation of the ordinate divided by the slope.

Sensitivities, LODs and LOQs of the sensors for method 1, method 2 and the *ex-situ* SbSPCE have been compared. To calculate them, solutions were measured at increasing concentrations from 1 to 200 μg/L for method 1 and 2 and increasing solutions from 25 to 100 μg/L for the *ex-situ* SbSPCE.

To carry out the comparative study, two sensors were prepared for each method. Figure 7 shows one of the two voltammograms obtained for method 1 and for method 2.

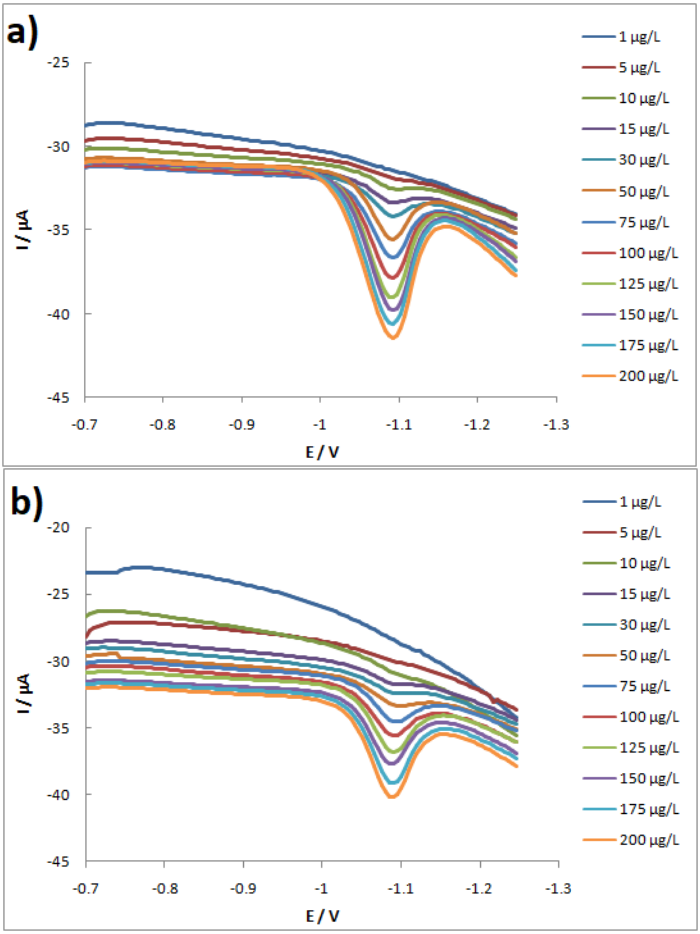


Figure 7. Differential pulse adsorptive stripping voltammograms of Ni(II) obtained using the sensor of **a**)method 1 and **b)** method 2. Series of plots correspond to increasing concentrations from 1 to 200 µg/L.

Once the voltammograms were obtained, the areas of the peaks were measured to construct the calibration line for the sensors of each method, taking into account both replicate measurements. Figure 8 shows the regression lines of the two sensors of each method with the standard deviations at each concentration point.

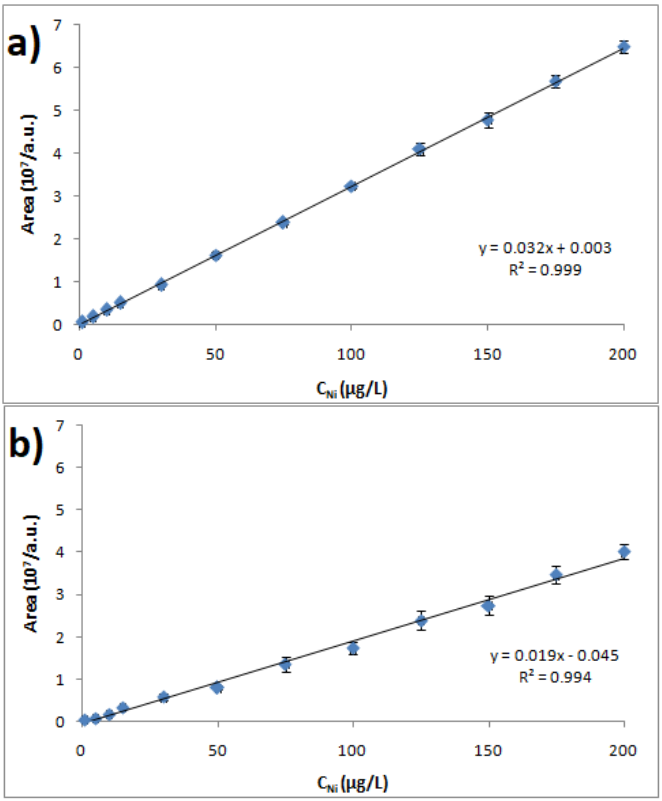
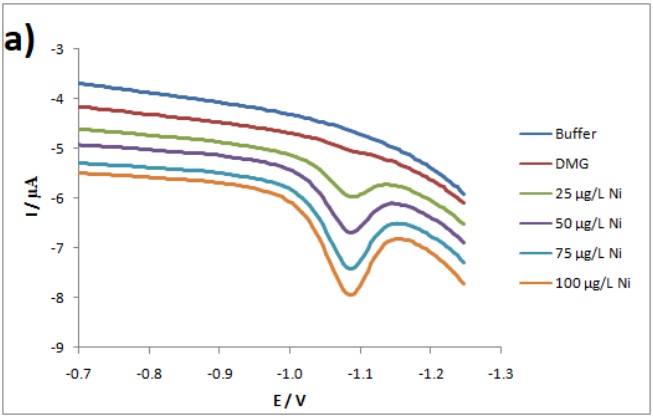


Figure 8. Calibration plot of Ni(II) for the sensors of **a)** method 1 and **b)** method 2.

The same experiment was performed for the ex-situ method SbSPCE. Figure 9 shows the voltammogram obtained and the calibration line constructed with the areas of the signal peaks.



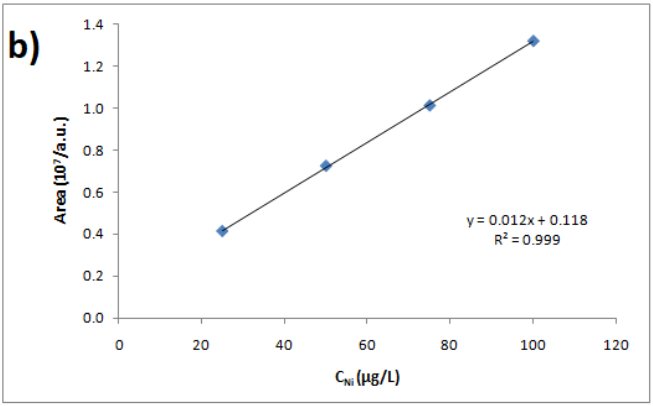


Figure 9. a) Differential pulse adsorptive stripping voltammograms of Ni(II) obtained using the *ex-situ* SbSPCE. Series of plots correspond to increasing concentrations from 25 to 100 µg/L. **b)** Calibration plot of Ni(II) on *ex-situ* SbSPCE.

To better compare between the *ex-situ* SbSPCE and the DMG modified SPE method, the voltammogram of each method is shown in Figure 10 for a 50 µg/L Ni(II) solution in ammonium buffer pH = 9.2. As seen, the DMG modified SPE method provides a greater signal than the ex-situ SbSPCE method.

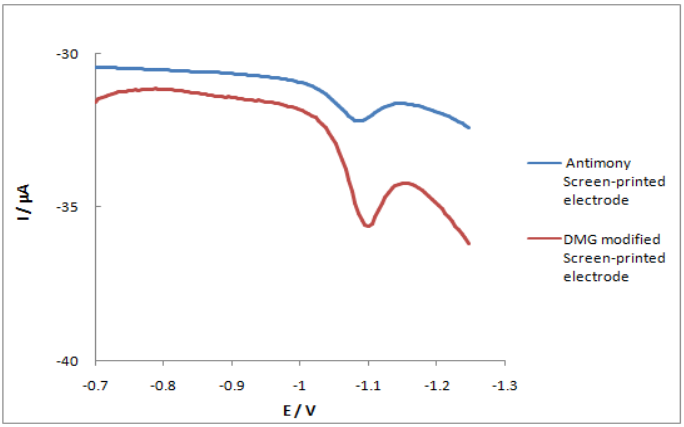


Figure 10. Differential pulse adsorptive stripping voltammograms of Ni(II) obtained using the *ex-situ* SbSPCE and the DMG modified SPE (method 1) with a concentration of nickel of 50 µg/L.

Then, in table 6, the equations of the calibration lines, the value of R2, the linear range (LOQ as the lowest value of the linear range) and the LOD for each method are shown. From the comparison between methods 1 and 2 in the preparation of the DMG modified SPEs can be determined that the sensors prepared with the method 1 have a higher sensitivity, better LOD and better LOQ. For this reason, method 1 was selected as the best one, and further experimental tests performed on the sensors will be with method 1, since method 2 is ruled out. Regarding the comparison between the ex-situ SbSPCE and the DMG modified SPEs strategy, it can be concluded that although they have a similar LOD and LOQ, the DMG modified SPE provides a greater signal in the measurements. Furthermore, with the added advantage that no extra reagent is required with the herein proposed approach.

Electrode	Regression	R^2	Linear range (µg/L)	LOD (µg/L)
Method 1	y = 0.032x + 0.003	0.999	7.6 - 200	2.3
Method 2	y = 0.019x - 0.045	0.994	23.6 - 200	7.0
Ex-situ SbSPCE	y = 0.012x + 0.118	0.999	7.0 - 100	2.1

Table 6. Calibration data for the determination of Ni(II) with *ex-situ* SbSPCE and DMG modified SPE using method 1 and 2.

6.2.3. Sensor reuse

The economic factor is a fundamental part of any project, and especially if aiming to achieve its usage by the stakeholders. For this reason, an important factor of the developed sensor is its useful life. During the experimental part of this work many sensors have been used for different experiments and up to 20 useful measurements of a single sensor have been obtained.

Reusability of the sensors also has been studied. To this aim, once the measurements have been performed with a sensor, this sensor was immersed in absolute ethanol for one minute. Since DMG is soluble in ethanol, by immersing the sensor in ethanol, the DMG layer is removed and the sensor can be re-modified. Analytical performance of the sensor upon this measure-reuse cycles was evaluated. Figure 11 shows a graph with the evolution of the measurements according to the days where the sensor has been cleaned with ethanol and has been re-prepared.

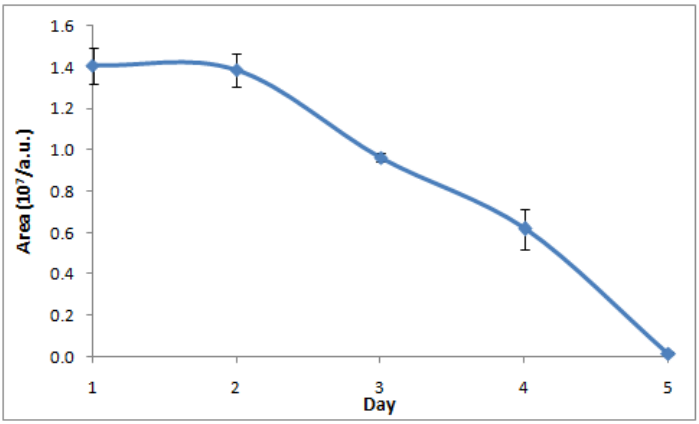


Figure 11. Graphic representative of the evolution of the measures as a function of the days of reuse.

Observing these data it can be determined that with the first cycle of washing and reuse the measures are not affected, but from the second cycle the values suffer a decline until no signal is observed. This decay in the performance of the sensor can be attributed to the disposable nature of SPE, which in most cases are not expected to be used for a large number of measurements.

6.2.4. Measurement after activation of the sensor

As already stated, if it aim to achieve the application of the developed sensor in real scenarios, it is much more interesting the possibility of having the sensors ready and use them after a while when necessary, rather than have to prepare them every time which is necessary. To assess if this option is viable, several sensors were prepared at once and they were tested in different days.

In the realization of this experiment, two sensors for each day were measured and a total of four measurements for each sensor were done. Figure 12 shows a graph with the evolution of the measurements over a month.

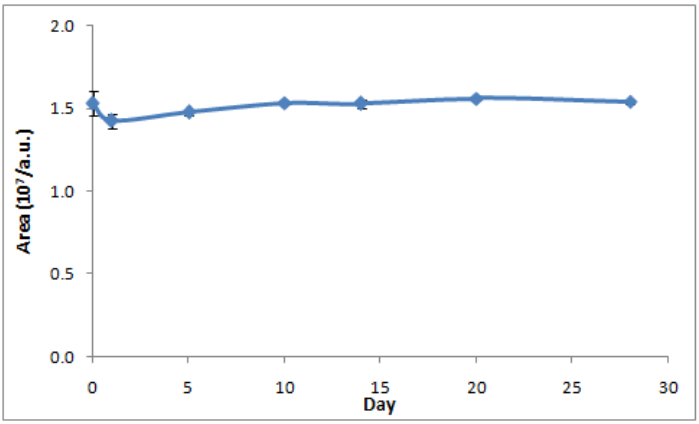


Figure 12. Graphic representative of the evolution of the measures as a function of the days when the sensors were activated and used.

Based on the data observed during the experiment it can be concluded that it is possible to chemically modify the sensors with DMG in advance and store them. Then, once we want to carry out some measurements, we just need to activate and use them. In this way, for at least one month, the results will still be conclusive.

6.3. DETERMINATION OF NICKEL IN A CERTIFIED WASTEWATER SAMPLE

Once the developed sensors were analytically characterized in terms of sensitivity, selectivity, LOD and LOQ, linear range, repeatability, reproducibility and durability, the last step was to apply them for the determination of Ni(II) by standard addition method and in triplicate in a certified sample of wastewater (reference SPS-WW2). Table 9 shows de composition of the certified wastewater sample (reference SPS-WW2).

In the performance of this experiment, the pH of the sample solution plays a very important role. The certified wastewater sample has a very acidic pH so when adding the ammonium buffer, a small amount of KOH solution will have to be added to obtain a pH of 9.2.

Once the sample solution is prepared, the first voltammetric measurement corresponding to the wastewater sample is performed. The following voltammetric measurements correspond to an increase in the concentration of Ni(II) of 25, 50 and 75 μg/L. Figure 13 shows representative AdSV voltammograms obtained in the analysis of the wastewater samples using DMG modified

SPE. Ni(II) behaves in the same way as in the stock solutions, with a well-defined peak for the metal ion obtained.

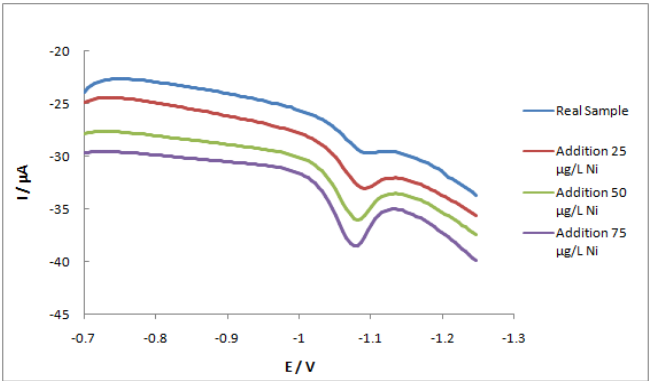


Figure 13. Differential pulse adsorptive stripping voltammograms of Ni(II) obtained using a DMG modified SPE. Series of plots correspond to certified sample of wastewater and to increasing concentrations from 25 to 75µg/L.

Once the voltammograms were obtained, the areas of the peaks were measured to construct the calibration line. The line is extrapolated to an ordinate value equal to zero and then, after undoing the initial dilution, the concentration of the certified sample of wastewater was obtained. Figure 14 shows the calibration line constructed with the areas of the signal peaks.

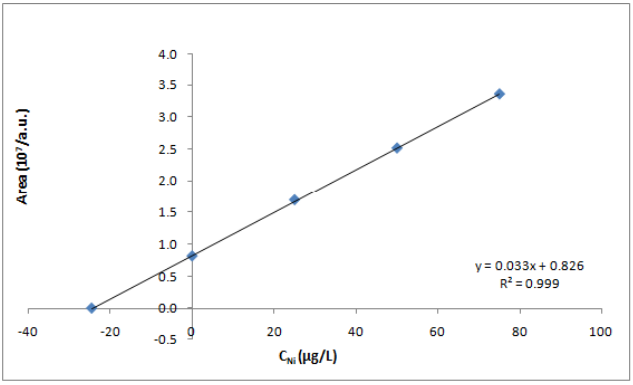


Figure 14. Standard addition plot of Ni(II) obtained using a DMG modified SPE. Series of plots correspond to certified sample of wastewater and to increasing concentrations from 25 to 75 µg/L.

Standard addition plot for Ni(II) (Figure 14) shows good correlation between the AdSV measurements carried out using DMG modified SPE. Ni(II) concentration data obtained from the AdSV determination of three replicates of the certified wastewater sample performed using the DMG modified SPEs are reported in Table 7. Average value of the three sensors was 5005.9 μg/L with an RSD of 0.005%, where as the real value of the certified sample of wastewater was 5000.00 ± 25. An excellent concordance between Ni(II) concentrations, as well as with the certified Ni(II) value in wastewater, was obtained.

Sensor	Regression	R^2	C_{Ni} (μg/L)
1	y = 0.033x + 0.826	0.999	5006.1
2	y = 0.036x + 0.851	0.999	5005.6
3	y = 0.034x + 0.851	0.999	5005.9

Table 7. Ni(II) concentration data obtained from the AdSV determination of three replicates of the certified wastewater sample performed using the DMG modified screen-printed electrode.

Thus, DMG modified SPE is postulated as a good and less toxic substitute for mercury electrodes for the determination of low level concentrations of Ni(II) in samples of environmental interest. Furthermore, the main advantages of our approach compared to other methods include its reagent-less nature, portability, durability and easy miniaturization, facilitating field detection in a rapid, reliable and inexpensive way, which should thus foster decentralized screening applications.

7. CONCLUSIONS

At view of the results obtained throughout this work, the following conclusions can be stated:

- A proven methodology has been established for the determination of Ni(II) with dimethylglyoxime (DMG) modified SPE..
- The developed sensors have been analytically characterized in terms of sensitivity, selectivity, LOD and LOQ, linear range, repeatability, reproducibility and durability.
- The developed sensor has been validated through the determination of Ni(II) in a certified wastewater sample obtaining a RSD of 0.005% and a relative error of 0.117%.
- Comparing the results obtained in this work with the results of the *ex-situ* SbSPCE method, it can be affirmed that the DMG modified SPE method provides better performance.

Based on the results obtained in this work, DMG modified SPE is proposed as a good alternative to more conventional mercury electrodes for the determination of Ni (II) in environmental samples.

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9. ACRONYMS

AAS	Atomic absorption spectrometry
AES	Atomic emission spectrometry
AdSV	Adsorptive stripping voltammetry
ASV	Anodic stripping voltammetry
ATSDR	Agency for toxic substances and disease registry
CME	Chemical modified electrode
DME	Dropping mercury electrode
DMG	Dimethylglyoxime
EPA	Environmental protection agency
ESPE	Graphite screen-printed electrode
GSPE	Graphene screen-printed electrode
HMDE	Hanging mercury drop electrode
IARC	International agency for research on cancer
IUPAC	International union of pure and applied chemistry
LOD	Limit of detection
LOQ	Limit of quantification
MFE	Mercury film electrode
RSD	Relative standard deviation
RSM	Response surface modeling
SbSPCE	Antimony film screen-printed carbon electrode
SMDE	Static mercury drop electrode
SPE	Screen-printed electrode
WHO	World health organization